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Short Communication

Discordance in the minimal inhibitory concentrations of ertapenem for *Enterobacter cloacae*: Vitek 2 system versus Etest and agar dilution methods

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SUMMARY

Our objective was to compare the ertapenem minimal inhibitory concentrations (MICs) for *Enterobacter cloacae* isolates categorized intermediate or resistant to ertapenem when measured with the Vitek 2 system, with the MICs for these isolates when measured by two methods performed in agar medium: the Etest and agar plate dilution method (APDM). Overall, 50 *E. cloacae* isolates were included in the study. The mean MIC of ertapenem was 2.92 ± 1.77 μg/ml according to the Vitek 2 system, 0.94 ± 0.84 μg/ml according to the Etest strips, and 0.93 ± 0.62 μg/ml according to the APDM. Furthermore, the MICs determined by the Vitek 2 system were higher than the MICs determined by the two other methods for 96% of strains. Lastly, according to the Etest strips and APDM, 42% of *E. cloacae* were susceptible to ertapenem. No carbapenemase was identified by the screening method used. Using the Vitek 2 system to determine ertapenem MICs for *E. cloacae* can have potential consequences in terms of additional carbapenemase-detecting tests and antimicrobial therapy. It would be interesting to determine if the Vitek 2 system is more effective for the detection of carbapenemase producers with low-level carbapenem resistance than the two methods performed in agar medium.

1. Introduction

Acquired carbapenemase-pro considerable threat to clinical Therefore, it is important to identify them in order to implement specific measures to prevent their dissemination. It is now suggested that carbapenemase-detecting phenotypic tests should be performed in isolates exhibiting even a small reduction in susceptibility to carbapenems, including ertapenem.[2]

Our objective was to compare the ertapenem minimal inhibitory concentrations (MICs) for *Enterobacter cloacae* when measured with the Vitek 2 system (bioMérieux, France) and when measured by methods performed in agar medium.

2. Methods

intermediate or resistant categories to ertapenem according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org; 0.5 < ertapenem MIC ≤ 1 μg/ml for the intermediate category, and ertapenem MIC > 1 μg/ml for the resistant category) were included.

MICs had been measured routinely by the automated dilution method with the Vitek 2 system. For the current study, the MICs were also measured in Mueller–Hinton agar by means of gradient diffusion testing with Etest strips for imipenem and meropenem (Oxoid Ltd, Basingstoke, UK) and for ertapenem and doripenem (bioMérieux, Marcy l'Etoile, France). Lastly, the ertapenem MICs were determined by agar plate dilution method (APDM; with ertapenem concentrations between 0.125 and 8 μg/ml). All agar plates were incubated at 37 °C for 24 h. The following strains were used for quality control (ertapenem MICs): *Escherichia coli* ATCC 25922, three *E. cloacae* isolates that had been characterized susceptible to ertapenem by Vitek 2 system and Etest strips in our

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Table 1
Enterobacter cloacae categorized intermediate or resistant to ertapenem by Vitek 2 system: beta-lactamase screening, ertapenem MICs (μg/ml) determined by the three different methods, and MICs of three other carbapenems determined by Etest strips

Strains	Ertapenem MIC (Vitek 2)	Ertapenem MIC (E-test)	Ertapenem MIC (Dilution in agar medium)	Imipenem MIC (E-test)	Meropenem MIC (E-test)	Doripenem MIC (E-test)	ESBL screening	Carbapenemase-detecting phenotypic test
1	2	1	0.5	0.12	0.06	0.032	Negative	Negative
2	2	0.75	0.25	0.25	0.12	0.094	Negative	Negative
3	1	0.5	0.5	0.5	0.12	0.094	Positive	Negative
4	4	1	1	0.5	0.12	0.094	Positive	Negative
5	4	1	1	0.25	0.12	0.094	Positive	Negative
6	>8	3	1	1	1	0.75	Negative	Negative
7	4	2	2	0.5	0.12	0.125	Negative	Negative
8	4	1.5	0.5	0.5	0.12	0.125	Positive	Negative
9	1	0.25	0.125	0.25	0.03	0.032	Positive	Negative
10	4	1.5	0.25	0.5	0.25	0.125	Negative	Negative
11	4	3	2	0.25	0.12	0.094	Positive	Negative
12	>8	1.5	0.5	0.25	0.12	0.64	Negative	Negative
13	2	0.75	0.5	0.5	0.12	0.047	Positive	Negative
14	2	1	0.5	0.5	0.12	0.094	Negative	Negative
15	2	1.5	2	0.5	0.12	0.094	Positive	Negative
16	1	0.75	0.25	0.25	0.12	0.094	Positive	Negative
17	1	0.125	0.25	0.25	0.03	0.016	Negative	Negative
18	2	0.25	1	0.25	0.12	0.064	Positive	Negative
19	2	1	1	0.5	0.12	0.094	Negative	Negative
20	4	0.75	0.25	0.25	0.03	0.023	Negative	Negative
21	4	0.5	0.25	0.25	0.06	0.047	Positive	Negative
22	2	0.75	1	0.25	0.06	0.064	Positive	Negative
23	1	0.25	1	0.25	0.06	0.047	Negative	Negative
24	2	0.38	1	0.5	0.06	0.094	Negative	Negative
25	4	1.5	2	0.25	0.12	0.094	Positive	Negative
26	0.75	0.75	2	0.5	0.12	0.125	Negative	Negative
27	2	1	1	0.25	0.06	0.047	Positive	Negative
28	4	0.38	0.5	0.25	0.12	0.032	Positive	Negative
29	8	4	1	0.25	0.12	0.094	Positive	Negative
30	1	0.25	0.5	0.5	0.06	0.032	Positive	Negative
31	4	2	2	1.5	1	0.5	Negative	Negative
32	1	3	1	0.5	0.25	0.19	Negative	Negative
33	2	1	1	0.25	0.12	0.125	Negative	Negative
34	4	0.25	2	0.5	0.12	0.064	Negative	Negative
35	4	1	1	0.12	0.06	0.064	Positive	Negative
36	1	0.75	0.25	0.12	0.03	0.016	Positive	Negative
37	4	1	2	0.25	0.12	0.125	Positive	Negative
38	2	0.25	0.5	0.5	0.06	0.064	Positive	Negative
39	2	0.75	1	0.25	0.06	0.047	Positive	Negative
40	2	0.38	2	0.5	0.12	0.094	Positive	Negative
41	2	0.25	0.5	0.25	0.06	0.047	Negative	Negative
42	4	0.5	0.25	0.25	0.06	0.032	Negative	Negative
43	4	1	1	0.25	0.12	0.064	Negative	Negative
44	4	0.5	1	0.25	0.12	0.094	Negative	Negative
45	2	0.19	2	0.5	0.06	0.125	Negative	Negative
46	4	0.12	0.125	0.06	0.008	0.004	Positive	Negative
47	2	0.38	1	0.25	0.06	0.064	Negative	Negative
48	2	0.25	1	0.25	0.06	0.047	Positive	Negative
49	4	0.25	1	1	0.12	0.25	Negative	Negative
50	1	0.25	0.25	0.25	0.06	0.047	Positive	Negative
E. coli ATCC 25922	≤0.5	0.125						
E. cloacae control 1	≤0.5	0.25						
E. cloacae control 2	≤0.5	0.25						
E. cloacae control 3	≤0.5	0.25						
OXA-48-producing *E. cloacae*	4	0.50						

MIC, minimal inhibitory concentration; ESBL, extended-spectrum beta-lactamase.

laboratory, and an OXA-48-producing *Klebsiella pneumoniae* for which the ertapenem MIC was 4 μg/ml with the Vitek 2 system and 0.50 μg/ml with the Etest strips in our laboratory.

In addition, all strains were tested for the presence of an extended-spectrum beta-lactamase (ESBL) by the combined disk method (Rosco Diagnostica A/S, Taastrup, Denmark).

Phenotypic testing for carbapenemases was performed by means of the double-disk synergy test (DDST) KPC + MBL Confirm ID Kit (Rosco Diagnostica A/S) using meropenem alone and in combination with dipicolinic acid, aminophenylboronic acid, and cloxacillin, with a disk of temocillin for screening OXA-48-producing strains.[3,4]

The comparison of the MICs determined with the different methods was performed using the Student's exact test or the non-parametric Kruskal–Wallis test, as appropriate.

3. Results and discussion

Fifty *E. cloacae* with a MIC of ertapenem >0.5 μg/ml determined by Vitek 2 system were isolated from 50 patients hospitalized on 20 different wards of the hospital. These isolates were from clinical or screening samples. An ESBL was identified in 52% of the strains. No carbapenemase was detected by the screening method used.

The mean MIC of ertapenem was 2.92 ± 1.77 μg/ml according to the Vitek 2 system, 0.94 ± 0.84 μg/ml according to the Etests, and 0.93 ± 0.62 μg/ml according to the APDM. The mean MIC determined by Vitek 2 system was significantly different to those determined by Etest strips and APDM ($p < 0.02$ and $p < 0.05$, respectively). Furthermore, the MICs determined by the Vitek 2 system were higher than the MICs determined by the two other methods for 96% of strains (Table 1). The mean ratio MIC Vitek 2 system/MIC Etest and MIC Vitek 2 system/MIC APDM was 5.2. The differences between the MICs were similar for ESBL-producing and ESBL-non-producing strains (Table 2). Lastly, according to the Etest strips and APDM, 42% of *E. cloacae* were susceptible to ertapenem (no strain was susceptible according to the Vitek 2 system). The mean MICs were 0.38 μg/ml for imipenem, 0.13 μg/ml for meropenem, and 0.11 μg/ml for doripenem.

Ertapenem has been described as the most appropriate carbapenem for detecting carbapenemase producers, especially those with low-level resistance to carbapenems.[4–6] In our experience, no *E. cloacae* identified as being intermediate or resistant to ertapenem with the Vitek 2 system has been a carbapenemase producer. The proportion of non-susceptible strains identified in the same population by the two other methods was only 58%. Therefore it appears that using the Vitek 2 system may lead to some excessive additional tests, such as the determination of MICs for other carbapenems and carbapenemase-detecting tests for third-generation cephalosporin-resistant *E. cloacae* strains. Moreover, the possibly falsely diagnosed non-susceptible strains may lead to an excessive use of other carbapenems that have a broader antimicrobial spectrum.

However, two considerations must be taken into account for the interpretation of these results. First, carbapenemase production is not the only mechanism that confers a reduced susceptibility to certain carbapenems. In addition, the inoculum effect can play a major role in the determination of the MIC.

Table 2
Ertapenem MICs (μg/ml) according to three different methods: comparison between *Enterobacter cloacae* producing ESBL and not

MIC	Production of an ESBL	Absence of ESBL
Vitek 2	2.8 ± 1.6	3.1 ± 1.9
Etest strips	0.91 ± 0.88	0.97 ± 0.82
Dilution in agar medium	0.89 ± 0.63	0.97 ± 0.62

MIC, minimal inhibitory concentration; ESBL, extended-spectrum beta-lactamase.

Finally, it is noticeable that the ertapenem MIC of the OXA-48-producing *K. pneumoniae* that was used as a control in our study was measured as 4 μg/ml (resistant) with the Vitek 2 system, and as 0.50 μg/ml (susceptible) by Etest. Therefore, it would be interesting to determine if the Vitek 2 system is more effective for the detection of carbapenemase producers with low-level carbapenem resistance than the two methods performed in agar medium.

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References

1. Cohen-Stuart J, Leverstein-Van Hall MA, on behalf of members of the Dutch Working Party on the Detection of Highly Resistant Microorganisms. Guideline for phenotypic screening and confirmation of carbapenemases in *Enterobacteriaceae*. *Int J Antimicrob Agents* 2010;**36**:205–10.
2. Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, et al. Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. *Clin Microbiol Infect* 2010;**16**:112–22.
3. Hartl R, Widhalm S, Kerschner H, Apfalter P. Temocillin and meropenem to discriminate resistance mechanisms leading to decreased carbapenem susceptibility with focus on OXA-48 *Enterobacteriaceae*. *Clin Microbiol Infect* 2013;**19**:E230–2. http://dx.doi.org/10.1111/1469-0691.12146.
4. Birgy A, Bidet P, Genel N, Doit C, Decré D, Arlet G, et al. Phenotypic screening of carbapenemases and associated β-lactamases in carbapenem-resistant *Enterobacteriaceae*. *J Clin Microbiol* 2012;**50**:1295–302.
5. Centers for Disease Control, Prevention. Guidance for control of infections with carbapenem-resistant or carbapenemase-producing *Enterobacteriaceae* in acute care facilities. *MMWR Morb Mortal Wkly Rep* 2009;**58**:256–60.
6. Nordmann P, Poirel L, Carrer A, Toleman MA, Walsh TR. How to detect NDM-1 producers. *J Clin Microbiol* 2011;**49**:718–21.